Liquid AP-MALDI: ion signal intensity and persistence at laser repetition rates between 1 Hz and 5 kHz

LIQUID AP-MALDI: ION SIGNAL INTENSITY AND PERSISTENCE AT LASER PULSE REPETITION RATES BETWEEN 1 Hz AND 5 kHz

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OVERVIEW

PURPOSE: To study the signal intensity and persistence of liquid AP-MALDI peptide signals at high laser pulse repetition rates on a Q-ToF.

METHODS: The inlet of the standard ESI source was modified to include a heated capillary and MALDI sample stage and the UV laser was operated at rates up to 5kHz.

RESULTS: Operating at laser pulse repetition rates up to 1kHz provided no loss in ion signal per laser shot. For 1 µL samples total experiment times were reduced from 1 hour (at 10Hz) to less than 1 minute per sample.

INTRODUCTION

Previous liquid AP-MALDI studies have shown that the technique is not only applicable for the generation of "ESI-like" multiply protonated ions from simple analytes such as peptides[1], but also for more complex classical proteomic experiments by LC-MS/MS[2]. Furthermore, analysis of larger molecular weight proteins such as bovine serum albumin have also been demonstrated by the technique[3].

The ion signals generated from liquid samples analysed by AP-MALDI MS are known to be significantly more stable and persistent than conventional solid MALDI samples due to the self-healing properties of the liquid sample format. Typically, UV lasers operating at 10Hz or 20Hz have been employed, continuously generating multiply protonated peptide ions from 1 µL loading of sample. Stable ion signals can persist for at least an hour.

In this work we investigate the effects on signal persistence, sample consumption, and ionization threshold laser pulse energy whilst operating the laser at repetition rates as high as 5kHz.

We found that liquid AP-MALDI MS experiments may be conducted 100 fold quicker than at 10Hz, with a corresponding increase in ion current.

METHODS

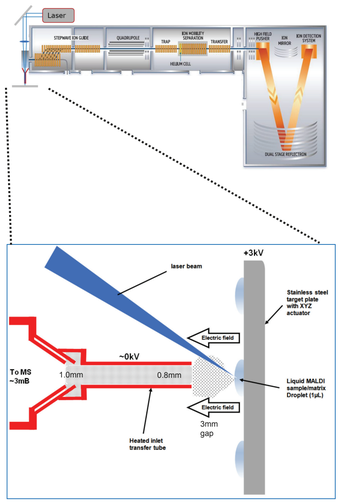

Figure 1. *Modified Synapt G2-Si system with the ESI source replaced by an experimental AP-MALDI source assembly including XY sample plate and heated inlet capillary*

An experimental AP-MALDI source assembly was fitted to a Synapt G2-Si (ion mobility enabled Q-ToF) (**Figure 1**). The standard ESI source housing was removed and a 0.8mm ID heated (250°C) ion transfer/desolvation inlet tube was fitted. An X-Y sample plate carrier was positioned 3mm in front of the ion transfer tube and samples were irradiated by a pulsed DPSS Nd:YLF laser (λ=349nm; ~8ns pulse duration). A potential of +3kV was applied between the MALDI target plate and the ion transfer tube. The laser energy per pulse was controlled using the diode pump current. The laser was focused using a plano-convex lens to an approximate diameter of 100µm with corresponding fluence of ~1kJ/m^2.

RESULTS

Initial experiments were carried out at a laser repetition of 10Hz (see **Figure 2**) whilst monitoring doubly protonated bradykinin molecules. The threshold laser pulse energy for ionization was approximately 6 µJ per laser shot. The laser energy per shot providing the greatest ion signal of was 18 µJ. Beyond 18 µJ signals diminished as the laser energy was increased.

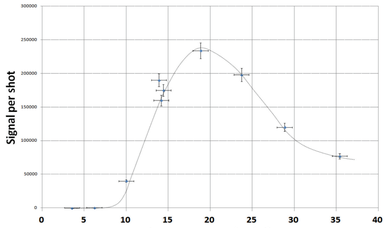

Figure 2. *Signal intensity (arbitrary units) per laser shot for bradykinin [M+2H]2+ as a function of laser energy at 10Hz laser pulse repetition rate. The ionisation threshold was at an energy of 6µJ and the maximum signal was at 18µJ.*

At 10Hz and14 µJ per shot, the shot-shot signal was reproducible and stable signals persisted for more than 90 minutes, corresponding to mean sample consumption rates of less than 20 nL per minute (or approximately 30 pL per laser shot). **Figure 3** illustrates the persistence of the **(a)** singly protonated bradykinin signal and **(b)** doubly protonated signal from the same sample droplet loading.

Sample Preparation:

•Sample: 10 pm/µL of bradykinin dissolved in H_2O with 0.1% formic acid.
•Matrix: 100 mg/mL 2,5-Dihydroxybenzoic acid in 70:30 ACN:H_2O + 60% glycerol (10 min. sonication)
•0.5 µL of sample (5pm) mixed with 0.5 µL of matrix on target.

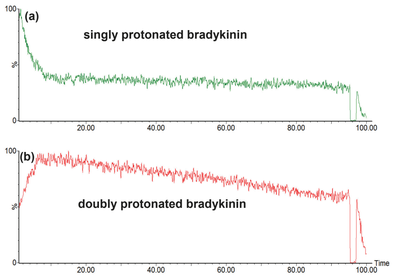

Figure 3: *A single micro-litre of sample/matrix solution produces (a) $[M+H]^+$ and (b) $[M+2H]^{2+}$ signals for more than 90 minutes when irradiated at 10Hz laser pulse repetition rate.*

For the higher repetition rate studies, the laser energy was also set at 14 µJ per shot. **Figure 4** shows the bradykinin $[M+2H]^{2+}$ signal at increasing repetition rates up to 1kHz acquired from a 1 µL sample loading.

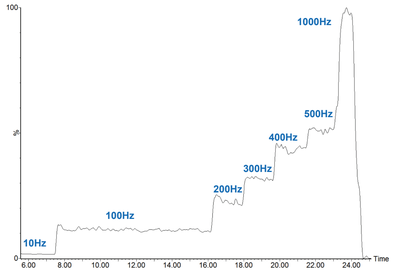

Figure 4: *Chromatogram of the doubly protonated bradykinin signal at increasing laser pulse repetition rates.*

From the data in **Figure 4**, the mean signal (for 20 shot spectra) at the different repetition rates was plotted in **Figure 5**. Between 1Hz and 1kHz an approximately linear relationship existed between analyte ion current (for the doubly protonated ion) and the laser pulse repetition rate.

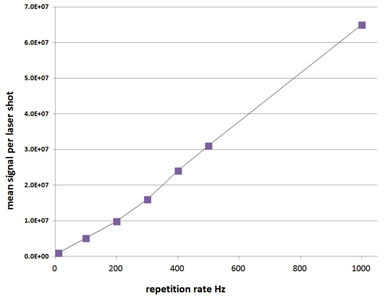

Figure 5: *bradykinin [M+2H]2+ signal (arbitrary units) as a function of laser pulse repetition rate up to 1kHz (at 14 µJ per shot).*

Systematic measurements beyond 1kHz, for more than a few minutes, were less reproducible and impractical due to the fast sample consumption rate. To avoid the excessive depletion limiting the measurements, larger sample volumes (~10 µL) were loaded (with the same analyte concentration).

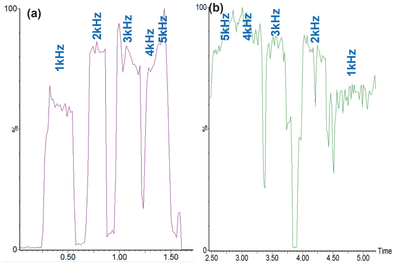

Figure 6: *Chromatograms of doubly protonated bradykinin at **a)** increasing repetition rates from 1kHz to 5kHz and **b)** decreasing repetition rates from 5kHz to 1kHz. Note the excessive signal fluctuation compared to **Figure 4.***

Figure 6a shows the signal as the laser pulse repetition rate was increased from 1kHz to 5kHz and then reduced from 5kHz to 1kHz in 1kHz steps. From the data in **Figure 6a and 6b** the mean signal for 1kHz to 5kHz was plotted in **Figure 7.**

For laser pulse repetition rates beyond 1kHz up to the maximum of 5kHz, the doubly protonated bradykinin signal became less reproducible and reached a plateau (i.e. the ion current stayed approximately constant beyond 1kHz). Further investigation will be necessary to understand why there are no benefits in operating faster than 1kHz.

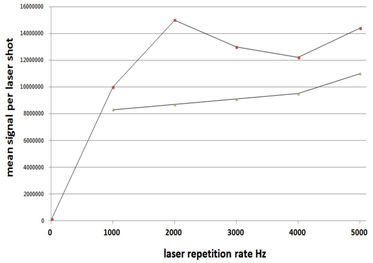

Figure 7: *The doubly protonated bradykinin signals generated between 1kHz and 5kHz were very approximately constant irrespective of laser pulse repetition rates.*

CONCLUSIONS

- For liquid AP-MALDI we found a significant speed advantage when operating at laser pulse repetition rates up to 1kHz.
- The ion count per unit time (ion current) is proportional to the laser pulse repetition rate up to 1kHz.
- There is a 100 fold speed advantage operating at 1kHz versus 10 Hz with no loss in total ions generated.
  ⇒ 1µL sample can be acquired in 30 or 40 seconds.
- Ion count per laser shot is reproducible up to 1kHz, beyond 1kHz ion count per shot is reduced and less consistent
- At laser pulse repetition rates between 1kHz and 5kHz the ion current did not increase linearly with repetition rate.

References

1 Pavel Ryumin, Jeffery Brown, Michael Morris, and Rainer Cramer. 2016. "Protein Identification Using a nanoUHPLC-AP-MALDI MS/MS Workflow with CID of Multiply Charged Proteolytic Peptides." *International Journal of Mass Spectrometry.* https://doi.org/10.1016/j.ijms.2016.12.006).

2 Cramer, R., Pirkl, A., Hillenkamp, F. and Dreisewerd, K. (2013) Liquid Ap-UV-MALDI enables stable ion yields of multiply charged peptide and protein ions for sensitive analysis by mass spectrometry. Angewandte Chemie-International Edition, 52 (8). Pp. 2364-2367.

3 Pavel Ryumin, Jeffery Brown, Michael Morris, and Rainer Cramer. Proceedings of ASMS 2016: Optimized Multiply Charged Ion Production By Liquid MALDI – From High Molecular Protein Ions To Bottom-Up Proteomics Using LC-MALDI MS/MS

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